basal half of trochanter, femur apically, tibia basally and apically, tibial spurs, and tarsus, and hind leg with coxa medially, trochanters, apical two-thirds of femur, tibia basally and medially, apical oblique half of basitarsus, and last two tarsal segments black. Abdomen black, first tergite, the second medially and anteriorly, the third, and venter except apex yellowish brown.

HEAD: Antenna 48-segmented; scape and pedicel shining and with longer pubescence than the flagellum; eye bare; ocelli elevated slightly, arranged in a flat triangle, the anterior member smaller than the lateral ones (Fig. 1); vertex wrinkled, punctate, shining, and slightly sloping towards frons; frons impressed, wrinkled, and with a distinct median longitudinal elevation; face rough, with a median, narrow triangular extension above; clypeus rugose, apical margin not notched medially; temple not bulging, narrower than the eye in dorsal view; galea short.

THORAX: Lateral face of pronotum rather plain; notaulices distinct, narrow, and finely foveolate; mesoscutum punctate; median lobe of mesoscutum plain without a longitudinal depression along each side of the median line; transverse fossa with five septa; mesopleuron punctate, upper groove flat and indistinct, lower groove oblique across the middle of mesopleuron, and posterior groove narrow and finely foveolate; metapleuron rather plain; propodeum flat and plain (Fig. 10), pleural carina distinct, areola elongate, flat and indistinct, transverse carina absent, spiracular carina very low, spiracular area acute posteriorly, and spiracle long ovate, directed obliquely in the center of the spiracular area. Wings with thin veins; stigma somewhat lanceolate; first abscissa of radius longer than that of the basal vein; the second abscissa about 2.5 times as long as the first; third and fourth abscissae of cubitus about equal; second abscissa of cubitus longer than the recurrent vein (Fig. 5); nervulus postfurcal by half of its own length; interanal vein absent. Tibia of middleleg thin, inner spur about as long as basitarsus; second and fifth tarsal segments about equal. Coxa of hind leg prominent; femur thin; tibia flattened, broadened toward apex, and with a smooth depression at the upper apical end; inner spur of tibia long, slightly over half as long as the basitarsus; hind basitarsus enormously developed, flattened, about as long as the hind tibia, and joined by the second tarsal segments ventrally at apex (Fig. 6); second tarsal segment slightly shorter than the fifth; hind tarsal claws pectinate basally.

ABDOMEN (Figs. 11, 12): Shorter than thorax; first tergite slightly longer than the second and third combined; first abdominal suture extending obliquely forward at the sides; second tergite slightly longer than the third medially; hypopygium about as long as the fourth and fifth segments of middle tarsus combined, obtuse in profile, and not nearly attaining apex of abdomen; ovipositor sheath very short, subexserted, and pubescent.

Type.—Female, Singapore, C. F. Baker, U.S.N.M., no. 57272.

PARASITOLOGY.—*Localization of radioactive antimony following multiple daily injections to a dog infected with* Dirofilaria immitis.[1]

DEAN B. COWIE, ALFRED H. LAWTON, A. T. NESS, FREDERICK J. BRADY, and GLEN E. OGDEN.[2] (Communicated by JOHN A. FLEMING.)

Antimony compounds have appeared to offer the most promise in the treatment of human filarid infections. In our studies it was found (1) that daily injections of several such compounds were effective in eradicating microfilariae of *Dirofilaria immitis* from naturally infected dogs. With regard to the fate of antimony in the tissues, we have reported (2) the distribution of radioactive antimony following a single intravenous administration of tartar emetic, sodium antimonyl xylitol, and an aqueous suspension of antimony trioxide. Since multiple daily injections of compounds at the dosage level of 0.8 milligram of antimony per kilogram of body weight were used in the experimental treatments, a knowledge

[1] Received March 16, 1945.

[2] From the Department of Terrestrial Magnetism of the Carnegie Institution of Washington and the Laboratories of Zoology, Chemistry, and Industrial Hygiene Research, National Institute of Health.

of the fate of the antimony after multiple injections was desired. In this paper a study with repeated injections of sodium antimonyl xylitol prepared from radioactive antimony is reported.

EXPERIMENTAL PROCEDURE

Radioactive antimony was prepared by the bombardment of antimony with deuterons in the cyclotron of the Carnegie Institution of Washington. The radioactive antimony was chemically separated from the other elements of the target and was recovered as nearly pure antimony trioxide. This was then synthesized into sodium antimonyl xylitol by the method described elsewhere (1). An aqueous solution of this chemical containing 10 milligrams of antimony per milliliter of solution was used throughout the experiment.

Dog no. 148 naturally infected with *Dirofilaria immitis* was given intravenously 0.8 milligram of antimony per kilogram of body weight as sodium antimonyl xylitol solution daily, except Sunday, for two weeks or a total of 12 injections. Blood samples were drawn immediately before each injection, 15 minutes after each injection, and 36 hours following the last injection. To these blood samples dry sodium citrate was added in the proportion of 10 milligrams per milliliter. Thirty-six hours after the last injection the dog was sacrificed and samples of 35 tissues were removed. The blood samples and the tissue specimens were weighed quickly after their removal, placed in a desiccator containing phosphorus pentoxide, and kept under reduced pressure at room temperature by means of a vacuum pump. After 16 hours of drying, tissues were reweighed and the amount of weight loss was determined. The samples were then ground in a mortar to a more or less homogeneous state.

Determinations of the antimony content of the blood and other tissues were made by measuring the number of disintegrations per second per unit weight of tissue powder with a Geiger-Müller counter and comparing this with a known standard. The standards were prepared by adding a known amount of the radioactive antimony to a sample of normal blood which

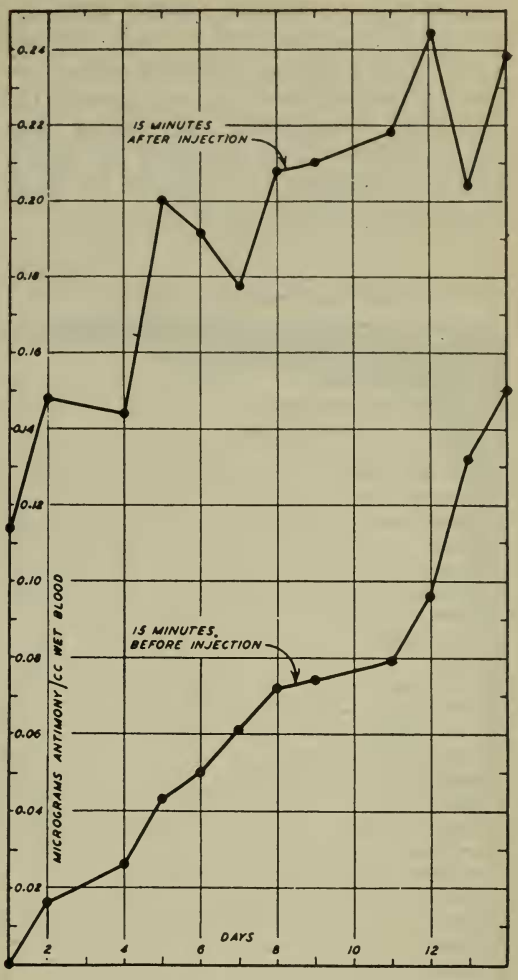

FIG. 1.—Antimony concentration in blood of dog no. 148, 15 minutes before and after injection.

was subsequently treated in the same manner as the samples containing the unknown quantities. An overall accuracy in these determinations of ± 1 per cent was demonstrated.

Microfilarial counts were made daily by a previously described method (3).

RESULTS

Figure 1 shows the blood content of antimony before each injection and 15 minutes after each injection. The antimony content is expressed in micrograms per gram of wet weight of blood. It will be noted that each injection causes the 15-minute postinjection level to exceed the previous

15-minute level, and the residual antimony in the blood at the end of each 24-hour period after injection remained above the previous residual level. An average of 0.112 microgram of antimony was cleared from each milliliter of the blood in 24 hours. With the cessation of treatment the antimony rapidly left the blood stream.

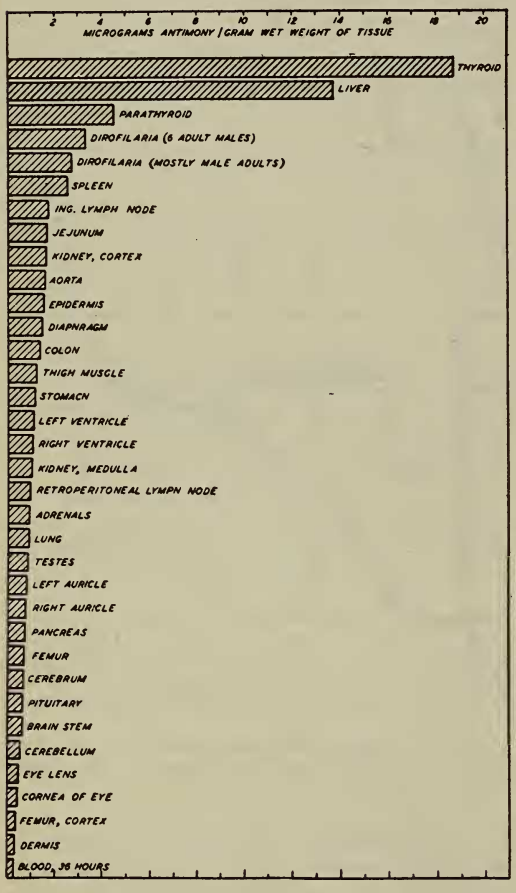

FIG. 2.—Antimony concentration in tissues of dog no. 148, 36 hours after last injection.

The dog in question became free of microfilariae after nine injections given over a 10-day period. Six live adult male *Dirofilaria immitis* were found in the right ventricle at autopsy. No live female parasites were recovered, but numerous fragments of recently dead and degenerated worms were removed from the pulmonary arterial tree. No other abnormalities were observed.

The antimony concentration of 35 tissues taken from this dog (no. 148) at autopsy 36 hours after the last treatment is given in Fig. 2 as micrograms per gram of wet weight of tissue. A wide variation in concentration values was recorded for the various tissues, ranging from 18.72 to 0.29 micrograms per gram of tissue. The thyroid gland had the greatest concentration of antimony. The liver also had a high antimony concentration, since each gram contained 13.75 micrograms. The parathyroid glands with 4.49, the filarids with 3.28, and the spleen with 2.60 micrograms of antimony per gram of wet weight were all higher than the highest blood level recorded in this experiment. It may be assumed that these tissues have a specific affinity for antimony. The other tissues are listed in the figure in the order of their antimony concentration.

DISCUSSION

In a previous paper (2) determination of the antimony in the blood after a single injection of tartar emetic and sodium antimonyl xylitol showed that there was an initial rapid decrease of the element during the first hour after injection followed by a slow removal for the next 4 to 16 hours with a slight secondary rise in the blood level at 24 to 36 hours. Of even more importance is the fact that the present experiment demonstrates continuous accumulation of the antimony in the blood. Parallel rates of accumulation are seen both 15 minutes and 24 hours after the injections. This shows that the repeated injection of 0.8 milligram of antimony per kilogram of body weight results in an accumulation of the element in the blood and that this dosage exceeds the clearance rate. Such a result adds support to the hypothesis (1) that a certain threshold of antimony must be reached before beneficial therapeutic results can be obtained. During the 24 hours preceding the elimination of circulating microfilariae, the highest recorded blood concentration was 0.218 microgram and the lowest was 0.096 microgram per gram of blood.

The irregularity of the curve showing the antimony level of the blood samples 15 minutes after treatment can be explained

on the basis that the rate of change was so rapid that an error of a minute or two in drawing the blood was reflected in the considerable change in the antimony level. In the case of the specimens taken 24 hours after the injection a time error of a minute or two made little difference in the results.

After single injections of tartar emetic, sodium antimonyl xylitol, or antimony trioxide, the liver contained the largest concentration of antimony. The thyroid and parathyroid tissues contained the next largest concentration and the adult *Dirofilaria immitis* ranked third. After 12 injections of sodium antimonyl xylitol, at the same dosage level as with the single injections, the thyroid gland contained the highest concentration of antimony, the liver was now second in antimony concentration, and the adult filarids remained third. A possible explanation for this reversal of the relative ranking of the thyroid gland and the liver is that the thyroid may continue its specific uptake of antimony whereas the liver may reach a point of equilibrium more quickly and the uptake and discharge of the antimony from the hepatic tissue may become equalized. In this way the thyroid gland finally exceeded the liver in the amount of antimony contained per gram of wet tissue.

There was an accumulation of antimony in all of the tissues studied and such tissues contained more antimony per gram of wet weight following multiple daily injections than they did following a single injection.

The finding of an element that is not known to enter into normal metabolic processes, such as antimony, in large quantities, in the thyroid gland was unexpected. These studies are being broadened so as to determine the relationship of this finding to the toxicology and therapeutic usefulness of antimony and other therapeutically active elements. This specific activity of the thyroid gland forms a broad basis for further studies in general physiology and pharmacology.

SUMMARY AND CONCLUSIONS

Twelve intravenous injections of sodium antimonyl xylitol in the amount of 0.8 milligram of antimony per kilogram of body weight daily, except Sunday, led to a continuous rise in the antimony level of the blood. The tissue levels of antimony were higher than those recorded following a single injection of this compound.

With the multiple injections of sodium antimonyl xylitol, the thyroid gland was found to contain the most antimony per unit weight and was followed in antimony concentration by the liver and the adult *Dirofilaria immitis*. Thirty-two other tissues showed a relatively small concentration of antimony, which was probably not of significance from a therapeutic standpoint.

It is believed that the observed accumulation phenomena offer evidence that a certain threshold of antimony must be reached before microfilariae of *Dirofilaria immitis* disappear from the peripheral circulation of infected dogs. It seems probable that a similar conclusion may apply in other helminth infections in which antimony is of value.

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